（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K753-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader(540-560 nm)

Detection range：15.61-2000 μmol/L

Elabscience® Lactose Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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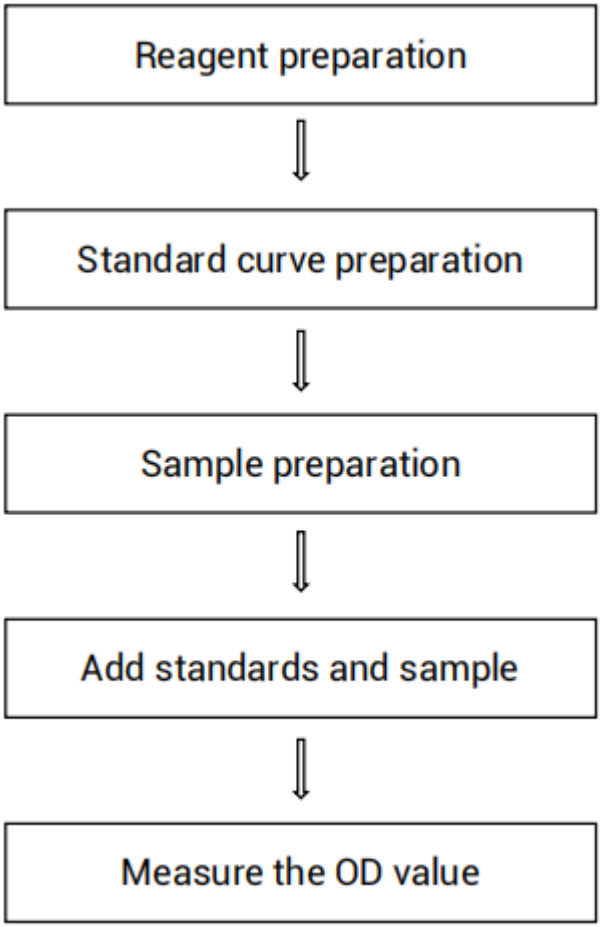
Assay summary



Intended use

The kit is suitable for detecting the content of lactose in milk, serum, plasma, animal tissues, and cells.

Detection principle

Lactose is a disaccharide composed of galactose and glucose, serving as the primary source of carbohydrates during mammalian development.
Lactose is catalyzed by galactosidase to produce galactose and glucose. The glucose is further converted by other enzymes, and the resulting product reacts with a chromogenic agent to form a colored substance with maximum absorption at 550 nm.The lactose content in the sample is determined by measuring the OD value at 550 nm and calculating from the standard curve.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Cell Lysis Buffer	50 mL × 1 vial	-20°C,12 months, shading light
Reagent 2	Buffer Solution	22 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Enzyme Reagent A	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 4	Enzyme Reagent B	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 5	Chromogenic Agent	1.1 mL × 1 vial	-20°C, 12 months, shading light
Reagent 6	50 mmol/L Standard	0.4 mL × 1 vial	-20°C, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

Microplate reader (540-560 nm, optimum wavelength: 550 nm), Incubator

Materials required but not provided

Distilled or deionized water, PBS (0.01 M, pH 7.4), 6 mol/L HCl, 6 mol/L NaOH, pH indicator paper

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② Enzyme Reagent A Working Solution preparation:
Dissolve one vial of Enzyme Reagent A with 550 μL of distilled or deionized water. Mix well to dissolve. Stable for 3 days when stored at -20°C protected from light.

③ Enzyme Reagent B Working Solution preparation:
Dissolve one vial of Enzyme Reagent B with 550 μL of distilled or deionized water. Mix well to dissolve. Stable for 3 days when stored at -20°C protected from light.

④ Working Solution preparation:
Before testing, please prepare sufficient Working Solution according to the test wells. For example, prepare 850 μL of Working Solution (mix well 750 μL of Buffer Solution, 50 μL of Enzyme Reagent B and 50 μL of Chromogenic Agent). The Working Solution should be freshly prepared before use. Stable for 2 h protected from light.

⑤ 2000 μmol/L Standard Solution preparation：
Before testing, prepare a sufficient 2000 μmol/L Standard Solution according to the test wells. For example, prepare 1000 μL of 2000 μmol/L Standard Solution (mix 960 μL of distilled or deionized water and 40 μL of 50 mmol/L Standard thoroughly). Stable for 7 days when stored at -20° C protected from light.

⑥ Standard curve preparation：

Always prepare a fresh set of Standards. Discard Working Standard Dilutions after use.

Dilute 2000 μmol/L Standard Solution with distilled or deionized water to a serial concentration. The recommended dilution gradient is as follows: 0, 100, 200, 400, 800, 1200,1600, 2000 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**100**	**200**	**400**	**800**	**1200**	**1600**	**2000**
2000 μmol/L Standard (μL)	0	10	20	40	80	120	160	200
distilled or deionized water (μL)	200	190	180	160	120	80	40	0

Sample preparation

milk and other samples with high protein content：

① Mix 200 μL of samples and 40 μL of 6 mol/L HCl, vortex thoroughly to mix. Incubate at 4°C (or on ice) for 5 min.

② Centrifuge at 12000×g for 5 min at 4°C. Collect supernatant and add 6 moL/L NaOH solution to adjust the pH to 6-7. (It is recommended to first add a small amount of NaOH for adjustment. For example, for 210 μL of milk sample treated with HCl, add 6 μL of NaOH for adjustment. Test 1 μL of the sample with pH indicator paper.)

Note: The sample may be diluted due to acid-base treatment, and the dilution factor should be calculated based on the specific amount of acid or base added.

Serum and plasma: detect directly.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 30 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C. Collect supernatant and keep it on ice for detection and detect within 2 days.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell sample

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Lyse 1×10^6 cells with 200 μL Cell Lysis Buffer. Place on the ice box and mix well every 5 min, lyse for 10 min.

④ Centrifuge at 10000×g for 10 min at 4°C to collect supernatant. Collect supernatant and keep it on ice for detection and detect within 2 days.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse kidney tissue homogenization	1
10% Mouse liver tissue homogenization	2-50
10% Mouse spleen tissue homogenization	1
10% Mouse lung tissue homogenization	1
Bovine serum	1
Rat serum	10-100
Horse serum	10-50
Human serum	1
Human milk	40-250
1×10^6 A549 cells	1

Note: The diluent is PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: Add 20 μL of Standard Solution with different concentrations to the corresponding wells.
Sample well: Add 20 μL of sample to the corresponding wells.
Control well: Add 20 μL of sample to the corresponding wells.

② Add 10 μL of Enzyme Reagent A Working Solution to standard wells and sample wells.

③ Add 10 μL of Buffer Solution to control wells.

④ Add 170 μL Working Solution to each well.

⑤ Shake the microplate for 5 seconds to ensure complete mixing. Incubate at 37°C for 20 min. Measure the OD value of each well at 550 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the corrected OD value.

3. Plot the standard curve by using corrected OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Liquid sample:

$$\text{lactose content (μmol/L)} = (\Delta A - b) \div a \times f$$

2. Tissue and Cell sample:

$$\text{lactose content (μmol/gprot)} = (\Delta A - b) \div a \div C_{pr} \times f$$

[Note]

ΔA: OD_{sample} - $OD_{control}$.

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human milk samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	124	815	1680
%CV	1.0	0.4	0.6

Inter-assay Precision

Three human milk samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	124	815	1680
%CV	3.2	5.0	6.5

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μmol/L)	124	815	1680
Observed Conc. (μmol/L)	124	570.0	1713.6
Recovery rate (%)	100	97	102

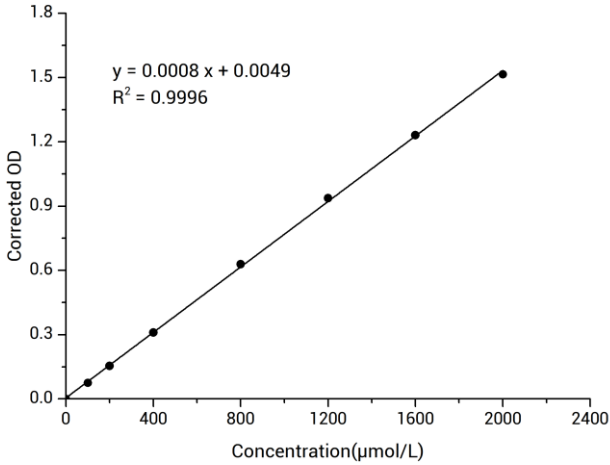
Sensitivity

The analytical sensitivity of the assay is 15.61 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	0	100	200	400	800	1200	1600	2000
OD	0.045	0.12	0.201	0.356	0.677	0.98	1.274	1.564
	0.047	0.121	0.199	0.356	0.674	0.985	1.280	1.559
Average OD	0.046	0.121	0.200	0.356	0.676	0.983	1.277	1.562
Corrected OD	0	0.075	0.154	0.310	0.630	0.937	1.231	1.516



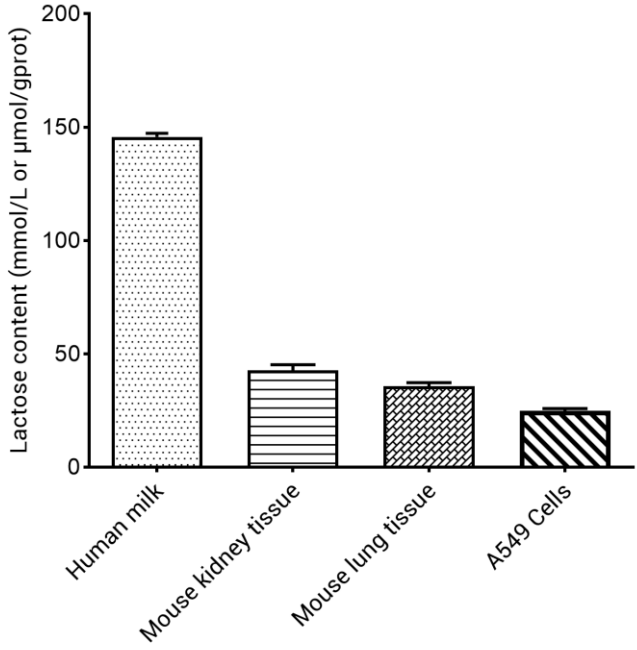
Appendix Π Example Analysis

Example analysis：

Take 20 μL of the 200-fold diluted human milk to the well of microplate. Proceed according to the operating steps. The results are as follows: standard curve: y = 0.0008 x + 0.0049, the average OD value of the sample well is 0.628, the average OD value of the control well is 0.043, ΔA=0.628-0.043=0.585, and the calculation result is:

lactose content (μmol/L) = (0.585 - 0.0049) ÷ 0.0008 × 200 = 145025 μmol/L

Detect human milk (dilute for 200 times), 10% mouse kidney tissue homogenate supernatant (the concentration of protein is 5.55 gprot/L), 10% mouse lung tissue homogenate supernatant (the concentration of protein is 4.93 gprot/L), 1×10^6 A549 cells (the concentration of protein is 2.61 gprot/L), according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.